I, Ruta S Deshpande, hereby submit this original work as part of the requirements for the degree of Master of Science in Environmental Engineering.

It is entitled:
Biodegradability of Diluted Bitumen (Dilbit)

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Biodegradability of Diluted Bitumen (dilbit)

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Abstract

The share of unconventional fuel sources (oil sands, shale gas etc.) in the oil market is growing in terms of production and usage. For the past 40 years, the United States has been importing petroleum products derived from Canadian oil sands. Nevertheless, in recent times, owing to the increased demand, the proposal of pipeline expansions along with two major spills (Kalamazoo, 2010 and Mayflower, 2013), these sources are again in the limelight. Oil sands yield bitumen which is the heaviest form of crude oil. For transportation purposes, bitumen is altered either by diluting or upgrading. When bitumen is blended with lighter hydrocarbons, diluted bitumen or dilbit is produced. Its physical and chemical properties are quite different from those of conventional oils.

Limited and inconsistent literature is available on the biodegradability of dilbit, thus the main aim of this research was to evaluate different aspects of its biodegradation to fill this knowledge gap. To achieve this goal various bench scale experiments were run in two phases.

In phase one, two tests were carried out at 5 and 25 ºC in freshwater media with two types of dilbits, western Canadian select (WCS) and Cold Lake Blend (CLB). Cultures isolated from sediments obtained after the dredging operations on Kalamazoo River spill were enriched and used as the inoculum. The physical and chemical characteristics of WCS and CLB differed slightly, whereas their composition was significantly different from that of a conventional crude oil. The degradation rates obtained for both dilbits as well as the extent of their removal were comparable, with faster rates for the higher temperature.

Phase two was a comparative study to assess biodegradation of dilbit and a conventional crude oil under several conditions. For this purpose, WCS dilbit and Prudhoe Bay Crude (PBC)
were tested. Additionally, microbial consortia obtained from two different hydrocarbon contaminated sites were used to evaluate the effect of microbial enrichments. Prior to the experimental set up, cultures obtained from Kalamazoo River (KMZ) where enriched on dilbit at 5 (cryo) and 25 (meso) °C, while culture isolated from Ohio River (AF) was enriched at only 25 °C. Although observed rates of alkane, polycyclic aromatic hydrocarbon (PAH), and total extractable hydrocarbon (TEH) removal were similar for dilbit and conventional crude oil, the latter degraded to greater extent. All three consortia showed diverse microbial community structure which greatly influenced degradation potential with varying temperature. Although complete alkane removal was achieved in all the treatments, the metabolism of PAH and TEH varied. The KMZ meso culture was the most efficient consortium as it metabolized most of the PAHs along with the biomarker hopane. The AF culture exhibited distinct behavior at the two temperatures with a longer assimilation time at the colder temperature, which was not the case at the warmer temperature.
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Patience does not mean to passively endure. It means to be farsighted enough to trust the end result of a process. It means to look at the thorn and see the rose, to look at the night and see the dawn. The lovers of God never run out of patience, for they know that time is needed for the crescent moon to become full.

- Elif Shafak, *The Forty Rules of Love*
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Chapter 1: Overview

1.1 Background

Globally, the utilization of conventional petroleum resources has steadily risen. Owing to the depletion of these fuels, alternative sources of energy has been sought. Although progress has been done in the field of renewable energies, a new breed of petroleum products is emerging as favored option. These sources, commonly known as unconventional petroleum products are not strictly defined but categorized according to their characteristics. Oil sands are one of the promising prospects among unconventional petroleum resources.

1.2 Oil Sands and bitumen

Oil sands are sandstones impregnated by bitumen, which is immobile at the reservoir temperature. These sand oils are geological mixtures of 83-85% of host sediment (sand, slit, clay or other mineral), 4-6% of water, and 10-12% of bitumen (Yang et al., 2011). Oil sand deposits are found throughout the world. The largest oil sand deposits are located in Alberta, Canada which also happens to be the world’s third largest oil reserve after Saudi Arabia and Venezuela. These sands are concentrated in the northeastern part of Alberta and are found majorly in Athabasca, Cold Lake, Peace River, and Wabasca regions.

In order to be used, bitumen needs to be recovered from the oil sands. This procedure depends on the occurrence of the deposits: if the deposits are found within approximately 75 m from the surface, then direct mining is used; for deposits buried deep underground, in-situ extraction methods like steam assisted gravity drainage process, cyclic steam simulation, or toe-to-heel air injection are required (Alberta Energy, 2016; CEPA, 2013). In Alberta, approximately 169.3 billion barrels of oil sand can be recovered with current technologies (Alberta Energy, 2016).
To obtain higher efficiencies in the production, new methods such as pulse technology and vapor recovery extraction are under development (Alberta Energy, 2016; Crosby S. et al., 2013).

The obtained bitumen is a viscous carbonaceous petroleum product, which is the heaviest form of crude oil. Bitumen is formed due to the biodegradation, water washing, and evaporation of conventional crude. Water washing eliminates the water-soluble light hydrocarbons, whereas the microbial degradation removes small chain compounds like \(n\)-alkanes and leaves a partially weathered product with higher concentrations of resins and asphaltenes (Lin et al., 1989). Because of these processes, oil undergoes physicochemical changes such as increased density and viscosity (because of higher concentrations of resins and asphaltenes), higher sulphur, acid and metal (nickel, vanadium) contents, and decreased API gravity (Crosby S. et al., 2013). Typically, bitumen has a density of 1,010 kg/m\(^3\) at 25 °C, viscosity of 760,000 cSt, and an API of 8. Thus for transportation purposes, bitumen needs to be altered. Fate of bitumen can be seen in Figure 1.1.

1.3 Diluted Bitumen (Dilbit)

At room temperature, bitumen is a solid petroleum deposit, which is thick like molasses. To be transported through a pipeline, bitumen must meet specifications such as a density of approximately 940 kg/m\(^3\) and a viscosity of 350 cSt at the pipeline reference temperature (7.5 – 18.5 °C). Bitumen can be upgraded by either coking or hydrocracking to make synthetic crude oil, which behaves very much like light crude oil. Bitumen can be blended with this synthetic crude oil in 1:1 proportion to produce synthetic bitumen or Synbit as well. After blended with diluents like naphtha, natural gas condensate, or light hydrocarbons bitumen is converted into diluted bitumen or Dilbit. Generally, ~75% of bitumen and ~25% of diluent are used (Canadian Energy Pipelines Association, 2013; Government of Canada, 2013). Both synbit and dilbit are classified as heavy crude oils.
Given that the composition of bitumen varies with the location of the deposits, and since different diluents can be used for dilution purposes; there is no uniformity in the quality or the composition of dilbit. Furthermore, the specifications for diluents are stated vaguely in the literature. It is suggested that diluents should have a density of 650-750 kg/m³, maximum viscosity of 2 cSt, and no more than 0.5 weight percent of sulfur. The most frequently used diluent are condensates, which are liquid by-products of natural gas extraction processes and contain mainly pentane and heavier hydrocarbons (Canadian Energy Pipelines Association, 2013; Crosby S. et al., 2013; Government of Canada, 2013). The Canadian Association of Petroleum Producer has formed a committee called Crude Oil Committee to regulate different aspects of this field. One of the sub-committees, the Crude Oil Quality Committee is working towards standardizing the condensate stream handled by Enbridge named as Condensate Blend (CRW) since the companies either use their own supply of lighter hydrocarbons or buy CRW for dilution (Alberta Innovates Energy an Environment Solution, 2011).

Dilbit (Cold Lake Blend and Western Canadian Select) composition in terms of weight percent of saturates, aromatics, resins and, asphaltenes (SARA) as compared to other crudes is represented in Figure 1.2. This figure shows that dilbit has higher proportions of resins and asphaltenes which makes it more viscous and denser as compared to other petroleum products. The average density for dilbit ranges between 824 and 941 kg/m³ at 15 ºC with average API gravity values in the range of 18-39 (POLARIS Applied Science, 2013).

The United States has been importing crude derived from oil sands from past 40 years (National Academies of Sciences, Engineering, and Medicine, 2016). Initially the crude was imported in the form of upgraded bitumen (i.e., synthetic crude oil) but, over the past few years, the demand of dilbit has increased: currently the United States imports around 1.2 million barrels
per day. This dilbit comes from Canada through three major pipelines namely TransCanada Keystone, Enbridge, and Kinder Morgan. To increase the supply of dilbit many pipeline expansion projects are being proposed. From Figure 1.3, we can see that these pipelines travel through the major part of U.S. mainland. Other than pipelines, oil sand products are also transported via rail and waterways.

Three major dilbit incidents were reported in past 10 years. On July 24th, 2007 a Trans Mountain Pipeline operated by Kinder Morgan Canada Inc. was punctured accidently by a construction crew along Inlet Drive in Burnaby, British Columbia, Canada. The pipeline was carrying crude called Albian Heavy, which is a blend of synthetic crude oil and bitumen. The discharged volume was 224,000 L that soaked into the surrounding soil, storm drains, sewer lines and eventually migrated to marine waters of Burrard Inlet. The spill affected 15 km of shoreline (Transportation Safety Board of Canada, 2007). Stantech Consulting Ltd. submitted a report (Stantec Consulting Ltd., 2012) on clean up and effect of this oil spill, which states that the emergency response and follow up remediation were effective in removing the oil from the environment and in limiting the short and long-term effects of the spill. Approximately 218,000 L of oil were recovered by skimming and booming as well as by flushing and removal from the affected shorelines. The Enbridge pipeline rupture released 3,320,000 L of dilbit that was released into Talmadge Creek and Kalamazoo River, near Marshall, Michigan on July 26th, 2010. This spill involved Cold lake and MacKay River blends. Presence of floating, submerged and sunken oil was reported. According to the report of the U.S. Environmental Protection Agency (USEPA, 2013a) approximately 700,000 L of oil still remains in the river submerged, bound to sediment for which dredging is being used. Dredging has damaged vast habitat for various species. Clean-up for this spill is still underway. Another dilbit spill occurred in March 2013 near suburban area of
Mayflower, Arkansas. The ExxonMobil’s Pegasus pipeline burst, causing the release of 800,000 L of Wabasca Heavy crude, which is a blend of bitumen and condensate. No reports are available on this spill as of now.

In the aforementioned spills, dilbit sank in the water, making the clean-up procedures more difficult and expensive. Although dilbit resembles heavy crude oil and is transported through the same pipelines, concerns regarding dilbit are growing. Due to the higher acid, sulfur, chloride, and abrasive solid content, dilbit is assumed to be more corrosive than heavy crude oils thus leading to higher pipeline failures. To date, there are limited studies on the physical chemical properties of dilbit, or on its fate and impact in the environment. Thus, it is difficult to either support or reject these claims on corrosion. There is also limited research on biodegradability in order to understand the behavior of dilbit in the environment.

1.4 Biodegradability of dilbit

It has been proposed that oil sands were formed due to the biodegradation and water washing of crude oils (Lin et al., 1989; Yang et al., 2011). Yang et al. (2011) did the chemical fingerprinting of Alberta oil sands and its related products by gas chromatograph coupled with flame ionization detector (GC-FID). They observed a large chromatographic hump of unresolved complex mixture (UCM), which represented the complex, non-biodegradable oil components. They postulated that the presence of UCM could be an indicator of extensive biodegradation of the original crude, which suggested that no further degradation was possible. Even more, in case of a dilbit spill, microbial removal is expected to be extremely low, as bitumen represents material that has already undergone degradation (Crosby S. et al., 2013). The United States Environmental Protection Agency (U.S. EPA performed a 28-day experiment at 30 °C to study the biodegradation of residual dilbit from the Enbridge oil spill, Kalamazoo River with microbes obtained from the
Kalamazoo River sediments (USEPA, 2013b). Total petroleum hydrocarbon (TPH) analysis and gas chromatography mass spectrometry (GC/MS) oil fingerprinting were done to evaluate biodegradation. The techniques described in the report could measure 60-75% of the TPH, where, they reported approximately 25% depletion in the measured TPH concentration. Nevertheless, in a study on the fate and behavior of two types of dilbit spilled in the seawater, King et al. (2014) reported biodegradation of dilbit after conducting a 13-day wave tank experiment. According to these authors, the presence of microorganisms in the seawater may have led to dilbit removal as a significant decrease in the concentrations of both alkanes and PAHs was observed. For total alkanes, they obtained first-order rate constants of 0.0011 and 0.0014 d\(^{-1}\) for Access Western blend and Cold lake blend, respectively. For PAHs, the corresponding rate constants were 0.0011 and 0.0005 d\(^{-1}\). Cobanli et al. (2015) studied the biodegradation of naturally and chemically dispersed diluted bitumen at different salinities. They used fresh surface seawater, 48-hour weathered dilbit, and chemical dispersant, Corexit 9500 at dispersant to oil ratio 1:25 in a set of 42-day experiments. At specific time points, they sacrificed the samples and extracted the oil with dichloromethane and analyzed them with GC/MS with analyte concentrations normalized to hopane. Similar results were obtained for treatments with and without the dispersant where during the 42-day period, alkanes were almost completely degraded for both salinities, whereas aromatics persisted. Additionally, salinity and the presence of dispersant did not affect the half-life of alkanes and aromatics.

1.5 Research Objectives

The literature available on biodegradability of diluted bitumen is limited and inconsistent. Thus the main aim of this study was to gain insight into the dilbit biodegradability. To investigate
different aspects of dilbit biodegradation, various bench scale experiments were carried out in two phases. Objectives for these experiments were to

- Characterize diluted bitumen products.
- Compare biodegradation of two types of dilbits.
- Study biodegradation of diluted bitumen and conventional crude oil under same conditions.
- Evaluate biodegradation rates of dilbits using different microbial consortia.
- Examine the influence of temperature on biodegradation.
- Investigate biodegradation of biomarker hopane.
- Temporal analysis of microbial community structure

1.6 Thesis Layout

This thesis has three more chapters. Chapter 2 summarizes the Phase One experiments where the main objective was to compare biodegradability of two types of dilbit. Chapter 3 reports results from Phase Two study. This includes examining effect of temperature on biodegradability of both dilbit and conventional crude oil using varied microbial consortia. Chapter 3 also discusses biodegradation of a common biomarker, hopane, and preliminary results from microbial community structure analysis (PCR analysis). Conclusions from this study and recommendations for future work are put forth in last chapter.

1.7 References


Figure 1.1: Fate of bitumen
Figure 1.2: Saturate-Aromatic-Resin-Asphaltene (SARA) composition for different oils

Figure 1.3: Canada-United States dilbit carrying pipeline network

Chapter 2: Biodegradability of Cold Lake Blend and Western Canadian Select Dilbit in Freshwater

Abstract

The principal goal of this study was to broaden the understanding of the biodegradability of diluted bitumen (dilbit). Cold Lake Blend (CLB) and Western Canadian Select (WCS) dilbit were evaluated for this purpose. Two batch experiments were set up at 5 and 25 °C and these experiments were carried out in freshwater media. Microbial consortia were isolated from the dredging operation on Kalamazoo River spill and were enriched on dilbit at 5 (cryo) and 25 (meso) °C. Dilbit was dispensed in flask containing sterile Bushnell Hass broth then inoculated with a bacterial culture and shaken on a rotary shaker at 200 rpm. Tests were run for 60 d at 25 °C and for 72 d at 5 °C. On every sampling day triplicates were sacrificed and residual oil concentration was determined. Oil compositional analysis (alkanes and PAHs) was performed by gas chromatography tandem mass spectrometry (GC-MS/MS). In addition, the total extractable hydrocarbons (TEH) were determined in the solvent extracts by GC equipped with a flame ionization detection. While the composition of the two dilbit oils showed some differences, their biodegradation trends were similar. Greater degradation rates and higher extent of biodegradation were achieved at 25 °C. Meso enrichment almost completely metabolized alkanes and aromatics whereas TEH concentration reduced by 70%. Biodegradation of the constructive biomarker hopane was also observed at higher temperature. Although cryo culture degraded most of the aliphatics and 70% of aromatics, TEH concentration dropped by just 20%.
2.1 Introduction

Oil sand deposits are distributed throughout the globe and the reservoirs found in Alberta, Canada are the largest and have the highest yield owing to the technologically most advanced production processes (Alberta Energy, 2016; CAPP, 2015b). Oil sand is a combination of sand, water, and the most viscous, heavily biodegraded form of petroleum crude bitumen. For transportation purposes, bitumen is altered either by upgrading or blending. When bitumen is blended with diluents like natural gas condensate, naphtha, or mixture of lighter hydrocarbons in approximately 7:3 proportion, diluted bitumen or dilbit is produced (Crosby S. et al., 2013). Although dilbit may resemble conventional heavy crude oil, there are significant differences between in their physical and chemical properties. Dilbit is richer in resins, asphaltenes, sulfur, and metals in addition to having a higher acid number (Meyer R. et al., 2007). The composition of bitumen varies significantly across as well as within the reservoirs (Larter and Head, 2014). Also, a variety of diluents are used for blending which results in inconsistent dilbit products.

The extraction of oil sand and derived products is escalating due to developments in technology and demand for unconventional hydrocarbon sources (CAPP, 2015c). According to Canadian Association of Petroleum Producers (CAPP, 2015a), their production will rise from 2.2 million barrels/day in 2013 to about 4 million barrels/day in 2030, because of this increase, many pipeline expansion projects (e.g., TransCanada Keystone XL, Enbridge Gateway, and Kinder Morgan Trans Mountain) have been proposed to boost the current capacity of the network (CAPP, 2015a). With the increased dilbit transportation, the risk of accidental spills has also grown. Three major diluted bitumen spills were reported in recent years: Kinder Morgan spill in Burnaby, Canada, Kalamazoo River Enbridge spill in Michigan, USA, and ExxonMobil’s spill in Mayflower, Arkansas, USA. Along with the preventive measures, preparedness to handle such...
mishaps is equally important and thus is essential to have comprehensive knowledge about the fate of diluted bitumen in environment.

Limited published studies are available in the literature about biodegradability of diluted bitumen (Cobanli S.E. et al., 2015; Crosby S. et al., 2013; USEPA, 2013; Yang et al., 2011). Cobanli et al. (2015), King et al. (2014) reported biodegradation of dilbit, whereas Crosby et al. (2013), USEPA (2013), and Yang et al. (2011) suggested otherwise. Hence, the main objective of this effort was to gain an insight into biodegradability of diluted bitumen to fill this research gap. Two types of dilbits were chosen to account for variability in dilbit compositions discussed earlier. These experiments were carried out in fresh water using microbial consortia obtained from the dredging operations on Kalamazoo River Enbridge spill. Since weather conditions can play a crucial role in biodegradation, two sets of experiments were run at 5 and 25 °C to simulate winter and summer conditions.

2.2 Methods and Materials

2.2.1 Chemicals and reagents

The U.S. EPA provided fresh Cold Lake Blend (CLB) and Western Canadian Select (WCS) dilbit for this study. Dilbits were stored according to MSDS requirements. Mineral salts, Dichloromethane (DCM), and hexane were obtained from Fisher Scientific (Pittsburg, PA, USA).

2.2.2 Media

Bushnell Hass was used for this experiment as fresh water media. It was prepared by dissolving magnesium sulfate (0.2 g/L), calcium chloride (0.02 g/L), monopotassium phosphate (1.0 g/L), dipotassium phosphate (1.0 g/L), ammonium nitrate (1.0 g/L), and ferric chloride (0.05 g/L) in distilled water. This broth was autoclaved at 120 °C for 15 min in batches of 1 L.
2.2.3 Microbial culture

Contaminated sediments from the dredging operation on the Kalamazoo River Enbridge Energy spill were used to obtain the mixed consortium. This enrichment was grown on Cold Lake Blend dilbit as the carbon source in Bushnell Haas broth for a month at both 5 (cryo) and 25 ºC (meso). Then the cultures were washed, concentrated tenfold, and frozen with 10% glycerol at -80 ºC for future use in the experiments.

2.2.4 Experimental setup and Procedure

Experimental design layout is summarized in Table 2.1 For the 25 ºC setup, there were 12 sampling events on 0, 2, 4, 8, 12, 16, 20, 28, 35, 42, 54, and, 60 d whereas, the 5 ºC sampling events were on days 0, 2, 4, 8, 16, 24, 32, 40, 48, 56, 62, and, 72. To account for abiotic losses, if any, kill controls (KC) were run in triplicate at each temperature and were sampled at the final event. Sodium azide (NaN₃) was used as sterilant at a concentration of 500 mg/L. On day 0, 75 µL of dilbit was dispensed into each shake flask containing 100 mL of sterile broth. All the samples were prepared using WCS or CLB and was spiked with 0.5 mL of the meso culture or the cryo culture for the experimental setup at 25 and 5 °C respectively. Flasks were placed on rotary shakers at 200 rpm in temperature controlled rooms. For any given sampling day, three flasks were sacrificed per dilbit and temperature. Bacterial activity was stopped by adding NaN₃ solution. For each replicate, oil was extracted with DCM. Obtained extracts were filtered through anhydrous sodium sulfate to remove any water and then concentrated under nitrogen gas. Solvent exchange was performed by adding hexanes to precipitate asphaltenes. These samples were then analyzed to measure residual alkanes and polycyclic aromatic hydrocarbons (PAHs).
2.2.5 Hydrocarbon Analysis

Remaining alkane and PAH concentrations were quantified using an Agilent 7890A Gas Chromatograph with an Agilent 7000 mass selective detector triple quadrupole and an Agilent 7693 series autosampler. This instrument was equipped with a DB-5 capillary column by J&W Scientific (30 m × 0.25 mm I.D. and 0.25 µm film thickness) and operated in splitless mode. Alkanes consisted of normal aliphatics ranging in carbon number from 10 to 35 as well as branched alkanes pristine and phytane. Aromatics included 2, 3 and 4-ring PAH compounds and their alkylated homologs [i.e. C_{0-4} naphthalenes (NAP), C_{0-4} phenanthrenes (PHE), C_{0-3} fluorenes (FLU), C_{0-3} dibenzothiophenes (DBT), C_{0-4} naphthobenzothiophenes (NBT), C_{0-3} pyrenes (PYR), C_{0-3} chrysenes (CHY)]. Multi Reaction Monitoring (MRM) mode was used for analyte detection. In addition, the total extractable hydrocarbons (TEH) in the solvent extracts were determined with a GC equipped with a flame ionization detector (FID) according to the EPA Method 8015C. Concentrations of all the individual alkanes and PAHs were summed up to measure total alkane and total PAH concentrations respectively.

As the hydrocarbon degradation pathways are ambiguous, degradation data was fitted to first order model using non-linear regression. The first order model can be represented as –

\[ C = C_0 \cdot e^{(-k \cdot t)} \]  

where \( C_0 \) is initial analyte concentration (µg/L), \( C \) is the analyte concentration (µg/L) at a particular time \( t \) (d), and \( k \) is the first order rate constant (d^{-1}). Non-linear regression analysis was performed using Microsoft Excel Solver. The first order rate constants were determined by minimizing the normalized mean square error from the observed sampling data. The average of analyte concentrations of three replicates from day 0 was used as initial concentration. Student’s \( t \)-test was conducted using SigmaPlot to test following null hypotheses (1) no difference exists in
biodegradability of two different dilbits and (2) temperature has no influence of biodegradability of diluted bitumen.

2.3 Results

2.3.1 Dilbit Characterization

Table 2.2 summarizes various properties of WCS and CLB dilbit. Density and sulphur content of both dilbits were comparable. While CLB had higher viscosity and total acid number, WCS showed slightly higher total alkane and total PAH values. The distribution of n-alkanes for both dilbits was similar, but differences in branched alkane composition were observed n-C17/pristane and n-C18/phytane ratios for WCS were lower as compared to CLB. WCS showed higher content of naphthalenes, while CLB was found to be richer in phenanthrenes and dibenzothiophenes. No differences were noted in the concentrations of other PAH groups.

2.3.2 Alkanes

Panels A and B in Figure 2.1 show time series concentration of total alkanes at 5 and 25 ºC respectively. WCS exhibited 22.5 % more total alkanes as compared to CLB. Both dilbits followed a similar trend of degradation at both the temperatures. Statistical analysis confirmed this observation as the difference between first-order rate coefficients was not statistically significant (for 5 ºC, \( p = 0.0754 \) and for 25 ºC, \( p = 0.0938 \)). Biodegradation rates for 5 ºC were from 5- to 7-fold lower than those for 25 ºC. Almost complete alkane removal was achieved by day 12 for 25 ºC whereas for 5 ºC, alkanes degraded completely by day 48. One of the reasons behind the lag in degradation at 5 ºC was the presence of branched alkanes. As seen in Figure 2.1 C, pristine and phytane persisted until day 48 for 5ºC treatment, while they were completely removed by day 8 at 25ºC. Degradation rates for WCS and CLB were statistically insignificant (for 5 ºC, \( p = 0.105 \) and for 25 ºC, \( p = 0.0604 \)). Pristane and phytane took significantly longer time to degrade at 5 ºC as n-
alkanes disappeared by day 8 while branched aliphatics persisted till day 48. At 25 ºC, the rate of iso-alkane degradation was lower than that for total n-alkane but they were removed only 8 days after complete removal of n-alkanes. Since n-alkanes degraded rapidly at 25 ºC, hence enough data collection was not possible for the calculation of first-order rate constants for individual analytes. Rate coefficients calculated at 5 ºC for individual n-alkanes provided inconclusive results on whether carbon number or type of dilbit had effect on biodegradation, as no pattern was observed in these values (Table 2.3). Branched alkanes degraded around 8 to 9 times faster at higher temperature.

2.3.3 Aromatics (PAHs)

Panels A and B in Figure 2.2 summarize the biodegradation data for total PAHs. Similar to alkanes, a t-test confirmed no differences between the biodegradation patterns for both dilbits at 5 and 25 ºC (p = 0.0876 and p = 0.139 respectively). At 5 ºC, an assimilation period of 4 d was noted, while the meso culture metabolized PAHs much faster. At the end of the run, around 97.5% of total PAHs degraded at 25 ºC, however only 70% was depleted at 5 ºC.

Figure 2.3 presents time series concentrations of 2-, 3-, and 4-ring compounds along with their alkylated homologues. Figure 2.4 shows percent removal whereas Figure 2.5 summarizes first order rate coefficients for individual PAH compounds. Biodegradation rates were considerably higher at 25 ºC for all the individual analytes. For all PAHs the rate of biodegradation was observed to be inversely proportional to number of rings as well as number of alkylation at both the temperatures. Individual PAH degradation rates for both dilbits were statistically insignificant (p > 0.05). In case of naphthalenes (Figure 2.3 A, B), virtually complete removal was achieved at 25 and 5 ºC, C\textsubscript{0·3}-NAP disappeared completely whereas C\textsubscript{4}-NAP concentration reduced by 80-85%. The meso culture almost completely degraded 3-ring aromatics PHE, FLU,
and DBT (Figure 2.3 D, F), while 35-40% residual of theses analytes was detected at lower temperature (Figure 2.3 C, E). This residue mainly comprised of C_{3,4}-PHE, C_{3}-FLU, and, C_{3}-DBT. NBT compounds persisted at 5 ºC (Figure 2.3 G) whereas at 25 ºC their concentration dropped by 92% (Figure 2.3 H). A significant decrease in 4-ring PAH concentration was reported at 25 ºC (Figure 2.3 I) while at 5 ºC, less than 10% of PYR + CHY degraded (Figure 2.3 J).

### 2.3.4 Hopane

A conservative biomarker, 17\alpha(H), 21\beta(H)-hopane was monitored with the intention to normalize the biodegradation data (Venosa et al., 1997). Nevertheless, the hopane concentration began to decline at day 12 for treatments at 25 ºC. This observation was made for both dilbits. Time series concentration of hopane at 5 and 25 ºC can be seen Figure 2.2 panels C and D. The meso culture degraded 93 and 98% of hopane in CLB and WCS, respectively (Figure 2.3 D), while no apparent change in concentration was noted at 5 ºC (Figure 2.3 C).

### 2.3.5 Total Extractable Hydrocarbon (TEH)

Along with the individual alkanes and aromatics, biodegradation of TEH was also studied and the results can be found in Figure 2.3 E and F. Approximately 700 mg/L of dilbit was spiked into the media at the start of the experiment and according to the initial TEH concentrations, they accounted for almost 90% of the added dilbit. Although cryo enrichment metabolized almost 100% of total alkanes and 70% of total PAHs, TEH concentration dropped by just 20% at 5 ºC. TEH degraded to higher extent (~70%) at 25 ºC. Half-lives for TEH were calculated for both temperatures and were found to be 24 d for both dilbits at 25 ºC, while at 5 ºC 192 and 120 d were measured for CLB and WCS, respectively. A t-test revealed that the half-lives at 5 ºC for the two dilbits were not statistically significant (p = 0.144).
2.4 Discussion

The biodegradation data for two types of dilbit as well as the effect of temperature on their biodegradability was reported. The observed properties for both dilbits were comparable to previously reported values (Alberta Innovates Energy an Environment Solution, September 2011; CEPA, 2013; Government of Canada, 2013; National Research Council, 2013; POLARIS Applied Science, 2013). Although dilbits herein studied contain bitumen of different origins, no major differences were observed in their properties. The extent of biodegradation as well as the pattern and rate of degradation were not statistically different.

The U.S. EPA (USEPA, 2013) carried out a biodegradation study on the residual dilbit from the Kalamazoo River Enbridge oil spill and noted approximately 25% reduction in total petroleum hydrocarbon concentration. King et al. (2014) reported biodegradation of dilbit after conducting a 13-day wave tank experiment but the observed rates of alkane and PAH degradation were minimal. Another study by Cobanli et al. (2015) investigated biodegradation of naturally and chemically dispersed diluted bitumen at different salinities. They observed complete degradation of alkanes while PAH remained constant even after 42 days of incubation. Unlike these previously reported findings, our study showed higher rates as well as greater extent of dilbit degradation.

Temperature influenced the biodegradation of dilbit. Rapid and almost complete degradation of alkanes, PAHs, and hopane was observed in the 25 °C treatments. The cryo culture metabolized most of the alkanes, naphthalenes and C_{0-2} homologues of 3-ring PAHs, but residual concentrations of C_{3,4}-PHE, C_{3}-FLU, C_{3}-DBT and 4-ring compounds were observed. At 5 °C, TEH content of dilbits barely depleted (~20%) whereas at 25 °C TEH concentrations dropped by 70%. Concentrations for kill control samples were similar to the time 0 sample point, which indicates that the reduction was solely due to biodegradation (Figure 2.6)
Mixed microbial consortia from the Kalamazoo River utilized dilbit at both temperatures, but to a different extent. The culture was enriched separately at two temperatures before setting up the experiments. Microbial community structure varies with temperature as psychrophilic and psychrotrophic microorganisms are active at lower temperatures, while thermophilic microorganisms dominate at warm temperature. Difference in biodegradation at the two temperatures can be explained by the variance in microbial community structure. Also many previous studies have reported prolonged biodegradation of crude oils at lower temperatures due to (1) limited microbial activity, (2) slower enzymatic metabolism, (3) poor solubility of hydrocarbons which results in reduced bioavailability, (4) increased viscosities and (5) reduced evaporation of toxic volatile petroleum products (Atlas and Bartha, 1972; Atlas, 1975; Campo et al., 2013; Frontera-Suau et al., 2002; Mogenesis and Schinner, 2001; Mulkins-Phillips and Stewart, 1974; Venosa and Holder, 2007; Zhuang et al., 2016).

This study provided elementary evidence to demonstrate that dilbit can be biodegraded. While no significant differences were observed between the two types of dilbit, the effect of temperature played a major role in the ease and extent of aliphatics and aromatics reduction. As biodegradation of any crude oil depends on various factors, in depth research is required to gain more knowledge regarding microbial degradation of diluted bitumen at various conditions.

2.5 References


19. POLARIS Applied Science, I. A compaison of the properties of diluted bitumen crudes with other oils. **2013**


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<th>Test</th>
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<th>Sample Replicates</th>
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Table 2.2: Physical and Chemical Properties of diluted bitumen

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<th>Properties</th>
<th>Cold Lake Blend (CLB)</th>
<th>Western Canadian Select (WCS)</th>
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<td>Total PAHs, µg/g of dilbit</td>
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**Table 2.3:** First order rate constants for individual $n$-alkanes at 5 °C

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Figure 2.1: Biodegradation of total alkanes (A, B) and Branched alkanes pristane (PR) + phytane (PH) (C, D) at 5 °C (A, C) 25 °C (B, D)
Figure 2.2: Time series concentration of Total PHAs (A, B), Hopane (C, D), and Total Extractable Hydrocarbons (TEH) (E, F) at 5 ºC (A, C, E) and 25 ºC (B, D, F)
Figure 2.3: Biodegradation of naphthalene (NAP) homologues (A, B), phenantheren (PHE) + fluorene (FLU) homologues (C, D), dibenzothiophenes (DBT) homologues (E, F), naphthenothiophene (NBT) homologues (E, F), and pyrene (PYR) + chrysene (CHY) homologues (G, H) at 5 °C (left panels) and 25 °C (right panels)
Figure 2.4: Percent removal of individual PAH compound
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Abstract

The objective of this study was to compare biodegradation of diluted bitumen (Western Canadian Select, WCS) with a conventional crude oil, Prudhoe Bay crude (PBC), under various conditions. Two laboratory experiments were set up, one conducted with a culture acclimated to dilbit (Kalamazoo River Enrichment, KMZ) and the second with a culture enriched on soil contaminated with hydrocarbons (Anderson Ferry Enrichment, AF). A 60-d long experiment was conducted at 25 °C, while the 5 °C experiment lasted 72 d. Microcosms were prepared with 100 mL freshwater media, 0.07 g of oil, and 0.5 mL of inoculum were added to each microcosm and they were kept on shakers in a temperature controlled rooms. On each sampling day, three replicates were sacrificed and the obtained samples were analyzed to quantify residual hydrocarbon [alkanes, polycyclic aromatic hydrocarbons (PAHs), and total extractable hydrocarbons (TEH)] concentrations. The rates of alkane, PAH and TEH degradation were comparable for the two oils, but the extent of degradation was greater for PBC owing to the higher concentrations of lighter alkanes. Lower degradation rates were achieved at the lower temperature. All the enrichments metabolized PBC as well WCS, but the nature and extent of the degradation was distinct. KMZ meso culture was the most effective among all, as it completely removed alkanes, PAHs, as well as hopane. AF enrichment performed differently at two temperatures; an acclimation period of 8 d was observed at 5 °C while there was no lag at 25 °C. KMZ cryo culture as well as AF culture at both temperatures degraded alkanes completely while they were not able to metabolize heavier fractions of the oil (C2-4 homologues of 3 ring compounds and 4-ring compounds).
3.1 Introduction

Crude oil is an important natural resource in the human history. However, to fulfill increasing energy demands, alternate resources have been investigated. Oil sands are a petroleum product that could supplement the growing global energy demand. Since they cannot be procured by the traditional methods, they are referred to as unconventional petroleum products (Gordon, 2012). Oil sands are sandstones impregnated by bitumen, which is immobile at the reservoir temperature. Alberta, Canada, has the largest oil sand basins and they are the third largest oil reserves after Saudi Arabia and Venezuela with 315 billion barrels of recoverable bitumen (Alberta Energy, 2016). Extracted bitumen is semisolid at room temperature thus, to facilitate its conveyance to refineries, bitumen is usually processed. It is either upgraded to produce synthetic crude or blended with diluents to yield diluted bitumen or dilbit. Bitumen is also mixed with synthetic crude to form synthetic bitumen or synbit (Crosby S. et al., 2013; Government of Canada, 2013).

Though diluted bitumen appears like heavy conventional crude oils, they have dissimilar physical and chemical properties. One of the major differences between conventional crude oil and dilbit is the composition. Dilbit has lower saturates and aromatics and shows higher relative proportion of resins and asphaltenes. It also has higher total acid number along with higher sulfur and metal content (Meyer et al., 2007; National Academies of Sciences, Engineering, and Medicine, 2016). After the dilbit spill in the Kalamazoo River, Michigan, USA, responders reported presence of floating, submerged and sunken oil (USEPA, 2013a) which shows that the behavior of dilbit in the environment and its weathering properties are quite different compared to a conventional crude oil.
While evaluating the fate of any substance in the environment, biodegradability is one of the major aspects to be considered. Several naturally occurring microorganisms in the environment are capable of metabolizing petroleum crudes. Although studies have reported on the biodegradation of conventional crude oils (Atlas and Bartha, 1972; Cao et al., 2009; Margesin and Schinner, 2001; Rojo, 2009), limited literature is available on the biodegradability of dilbit. Given that bitumen is formed due to the extensive biodegradation of conventional crudes, Crosby S. et al. (2013) and Yang et al. (2011) suggested that no further degradation is possible. Batch experiments set up by the U.S. EPA (2013b) to study biodegradation of residual dilbit from Kalamazoo spill reported 20% removal of total petroleum hydrocarbons over a period of 28 d. The Agency concluded that dilbit is not further biodegradable. However, Cobanli et al. (2015) studied biodegradation of naturally and chemically dispersed dilbit and observed complete removal of alkanes and persistence of aromatics. King et al. (2014) also reported biodegradation of dilbit after conducting a 13-day wave tank experiment but the rates of alkane and PAH degradation were negligible.

As discussed earlier, dilbit exhibits unique properties compared conventional crude oil. However, no conclusive work has been reported which compares biodegradability of these crudes. Thus, the objective of the present work was to assess biodegradability of diluted bitumen and a conventional crude oil under various conditions. For that purpose, experiments were set up at two different temperatures, 5 and 25 °C and both oils were metabolized by microbial consortia obtained from two different hydrocarbon impacted areas.
3.2 Methods and Materials

3.2.1 Chemicals and Reagents

For this comparative study, Prudhoe Bay Crude (PBC) and Western Canadian Select (WCS) were used to represent a conventional crude and dilbit, respectively. Both WCS and PBC were provided by the U.S. EPA. Mineral salts, dichloromethane (DCM), and hexane were acquired from Fisher Scientific (Pittsburg, PA, USA).

3.2.2 Media

Bushnell Hass broth was used as fresh water media. It was prepared by dissolving the required amounts of mineral salts in distilled water. The concentrations of each salt (expressed in g/L) were magnesium sulfate (0.2), calcium chloride (0.02), monopotassium phosphate (1.0), dipotassium phosphate (1.0), ammonium nitrate (1.0), and ferric chloride (0.05). This solution was autoclaved at 120 °C for 15 min in batches of 1 L. The pH for this media was ~7.

3.2.3 Microbial Enrichment

Cultures were enriched on hydrocarbon impacted sediments isolated from two different locations. One set of sediments was collected from the Ohio River, downstream of fuel tanks at Anderson Ferry on the west side of Cincinnati, Ohio, USA. The other batch of sediments was obtained from the dredging operations following the Kalamazoo River Enbridge Energy Spill. The cultures were named after the locations from where they were acquired, the former enrichment was denoted as Anderson Ferry (AF), while latter one was named as Kalamazoo River (KMZ) culture. AF and KMZ were enriched on Alaskan North Slope (ANS) 521 and dilbit, respectively as the carbon source in Bushnell Haas broth for a month. KMZ was enriched at both 5 (cryo) and 25 °C (meso), however AF was grown only at 25 °C. After 30 days of incubation, cultures were centrifuged, washed with saline and mixed with 10% glycerol before storing at -80 °C. Obtained
cultures were undefined mixture of microbial consortia. On the day of experimental set up, enrichment stocks were thawed to room temperature and re-suspended in sterile saline before use.

### 3.2.4 Microcosm Set up

Experiments were set up to examine biodegradation of two types of crude oils at 5 and 25 °C using microbial consortia obtained from different locations. The experimental layout is summarized in Table 3.1. Sampling days at 25 °C occurred at days 0, 2, 4, 8, 12, 16, 20, 28, 35, 42, 54, and 60. The 5 °C experiments were run for longer period of time and the samples were analyzed on days 0, 2, 4, 8, 16, 24, 32, 40, 48, 56, 62, and 72. To account for any possible abiotic losses, triplicate killed controls (KCs) containing 500 mg/L of sodium azide were also included and sampled at the end of the run.

### 3.2.5 Procedure

Each flask containing 100 mL of broth was spiked with 0.07 g of oil and was inoculated with 0.5 mL of the culture. The KCs were prepared by adding 500 mg/L of the sterilant sodium azide. These microcosms were then placed on a rotary shaker and mixed at 200 rpm in temperature controlled rooms for the duration of the experiment.

### 3.2.6 Oil extraction and analysis

On every sampling day, three flasks were sacrificed per treatment. Oil was extracted with DCM. Obtained extracts were filtered through anhydrous sodium sulfate to remove any water and then concentrated to reduce the volume under nitrogen. Residual hydrocarbons were measured with an Agilent 7890A Gas Chromatograph with an Agilent 7000 mass selective detector triple quadrupole. The chromatograph was equipped with a DB-5 capillary column by J&W Scientific (30 m × 0.25 mm and 0.25 µm film thickness) and a split/splitless injection port operated in the splitless mode. Multi Reaction Monitoring (MRM) mode was used for analyte detection. The
analytes included 28 alkanes ranging in carbon number from \( n\)-C10 to \( n\)-C35 plus pristane, phytane. Aromatics consisted of the 2-, 3-, and 4-ring polycyclic aromatic hydrocarbons (PAHs) [naphthalenes (NAP), phenanthrenes (PHE), fluorenes (FLU), dibenzothiophenes (DBT), naphthbenzothiophenes (NBT), pyrenes (PYR), and chrysenes (CHY)] along with their alkylated homologs. Concentrations of individual analytes were added together to calculate total alkanes and total PAH content. Along with these hydrocarbons, the conservative biomarker hopane was also monitored. Total extractable hydrocarbons (TEH) were determined with a GC flame ionization detector according to the EPA Method 8015C.

Biodegradation of all the hydrocarbons was assumed to be first order and first order rate coefficients were determined by non-linear regression using Microsoft Excel Solver. An analysis of variance (ANOVA) was conducted using SigmaPlot to test the following null hypotheses: (1) no differences exist in the biodegradability of dilbit and conventional crude oil, (2) all the enrichments metabolize crude in a similar fashion, and (3) temperature does not affect biodegradation of crude.

3.3 Results

3.3.1 Alkanes

Expectedly, PBC showed higher aliphatic content whose concentration was nearly 6-fold greater than that for WCS. Figure 3.1 summarizes biodegradation of total alkanes: KMZ (closed symbols) culture nearly completely eliminated total alkanes at both the temperatures and for both the crudes. Biodegradation rates were higher at warmer temperature, and the extent of removal exceeded 99% by day 8 while it took 40 days to achieve the same level of degradation at 5 °C. Like KMZ, AF (open symbols) was able to metabolize aliphatics completely at 25 °C, but the degradation rates were lower for this enrichment. An acclimation period of 8 days was observed
during the AF treatment at 5 ºC (Figure 3.1 A, C). At the end of the run, almost 98% of total alkanes disappeared, and residual alkanes mainly were iso-alkanes. Biodegradation of branched alkanes is shown in Figure 3.2. Rapid and complete removal of pristane and phytane was achieved at 25 ºC. After a lag of 8 days, iso-alkanes started depleting at the lower temperature and trace amounts of branched alkanes were noted at the end of the experiment. The variability in three replicates was much higher at 5 ºC. At both the temperatures, removal was much faster using KMZ enrichment. Degradation patterns for both the oils were similar for AF ($p = 0.5$) as well as KMZ treatments ($p = 0.42$).

### 3.3.2 PAHs

Figure 3.3 shows biodegradation of total PAHs. The pattern of PAH degradation varied for each treatment. The total PAH fraction of the conventional crude oil was almost twice of the dilbit aromatic content. The KMZ meso culture metabolized almost 98% of the total PAHs for both oils (closed symbols in Figure 3.3 B, D) whereas, in 5 ºC experiment, the extent of biodegradation by KMZ culture was observed to be 75 and 85% for WCS and PBC, respectively (closed symbols in Figure 3.3 A, C). At higher temperature, AF culture degraded PAHs rapidly until day 20, after which no change in concentration was observed for either oil. About 40% of the initial PAH load remained in WCS while 20% of residual PAHs were found in PBC (Open symbols in Figure 3.3 B, D). Lower degradation rates were achieved at 5 ºC. After the acclimation of 4 days, PAHs started depleting at the lower temperature. AF enrichment was able to metabolize 50% PAH content of WCS and 78% in case of PBC (Open symbols in Figure 3.3 A, C).

PAH distribution for the two oils was quite different; naphthalenes accounted for 58% of total PAHs in PBC, but only 35% in WCS. Also, quantities of 3- and 4-ring compounds in PBC were less than those for WCS. Biodegradation of individual PAHs and their alkylated homologues
for both the crudes are represented in Figure 3.4 (5 ºC treatments) and Figure 3.5 (25 ºC treatments). Naphthalenes diminished faster and to greater extent as compared to other PAHs. C_{0,2}-NAP were degraded completely (Figure 3.6). Both cryo and meso KMZ enrichments were able to metabolize 100% of C_{3}-NAP and almost 90-95% of C_{4}-NAP. Nevertheless, AF culture could not complete degradation of C_{3,4}-NAP for either of the crudes. Higher removal of the 3-ring compounds, PHE and FLU as well as DBT was noted at 25 ºC for the KMZ treatments. For the AF treatments at 25 ºC, the concentration dropped till day 20 after which there was no apparent change. Almost 33 and 57% of the initial mounts were seen in PBC and WCS samples, respectively. Such fractions mainly comprised of C_{2,4}-PHE, C_{2,3}-FLU, and C_{1,3} DBT. At 5 ºC, AF metabolized C_{0,1}-PHE, C_{0}-FLU, and C_{0}-DBT hydrocarbons, while treatments with KMZ cryo culture showed disappearance of C_{1,2}-FLU, C_{0}-DBT in addition to aforementioned compounds. No significant change was observed in concentrations of heavier hydrocarbons like NBT, CHY, and PYR in treatments involving AF enrichment as well as KMZ cryo culture. However, KMZ meso culture was able to assimilate 80% of the 4 ring compounds in both the oils.

3.3.3 TEH

Along with the individual alkanes and aromatics, TEH concentrations were also quantified. TEH represents hydrocarbon compounds with carbon number ranging from 10 to 28 (EPA, 2000), In this case, they accounted for ~90% of the total oil added to each microcosm. Their biodegradation data is reported in Figure 3.7. The trend of TEH depletion was similar to the total PAH degradation of the respective treatments. TEH degraded to greater extent for all the treatments utilizing PBC. At 25 ºC, 25 and 53% of TEH loss was observed for WCS and PBC respectively while using the AF culture (open symbols in Figure 3.7 B, D). In the case of KMZ meso culture (closed symbols in Figure 3.7 B, D), 70 and 80% of WCS and PBC was degraded.
The rate of TEH removal was slower at the lower temperature. The decrease in TEH concentrations by KMZ cryo and AF culture at 25 °C were similar [Figure 3.7 A, C (closed symbols) and B, D (open symbols)] and these values were slightly higher than the removal achieved by AF culture at 5 °C (open symbols in Figure 3.7 A, C).

3.3.4 Hopane

It is a common practice in biodegradation studies to normalize analyte concentrations by the concentration of non-biodegradable biomarkers like hopane (Venosa et al., 1997). For the same purpose, hopane concentrations were measured. Hopane remained constant for all the treatments with AF consortia (open symbols in Figure 3.8). Similar results were observed for KMZ cryo culture. The KMZ meso culture, however, metabolized hopane (closed symbols in Figure 3.8 B, D) and it was completely eliminated by the end of the run for PBC as well as WCS.

3.3.5 Microbial Community Structure analysis

Consortia used in these experiments were isolated from two different locations and were enriched on two different oils at different temperatures prior to the inoculation of the microcosm. Therefore, microbial community structure analysis was done to characterize these enrichments, and DNA extracts were sequenced for this purpose. This analysis provided the composition of the three cultures KMZ meso, KMZ cryo and AF.

Table 3.2 summarizes the distribution of these enrichments at the phylum and genus levels, where taxa are listed in order of decreasing abundance. On the phylum level, no significant difference was observed. Proteobacteria was the dominating phylum in all the cultures, additionally, Actinobacteria and Bacteroidetes were also present in both the KMZ cultures. Oil degrading microbial community on the genus level was markedly different between the KMZ cultures and AF. Acinetobacter (72%) was the dominant genus in AF microbial community,
whereas high abundance of *Pseudomonas* (13%, 17%), *Rhodococcus* (22%, 26.5%), and *Hydrogenophaga* (15%, 12%) was observed in KMZ cryo and meso cultures. Though several other genera were present in consortia, their abundance was very low as compared to the above mentioned genera.

### 3.4 Discussion

This study reported the biodegradation of dilbit and a conventional crude oil under different conditions. The two oils exhibited different hydrocarbon profiles, with PBC comprising of higher concentrations of lighter hydrocarbons such as alkanes and naphthalenes, and WCS is rich in heavier PAHs. Owing to this composition, PBC degraded to greater extent as compared to dilbit. Biodegradation rates of alkanes, PAHs and TEH for all the treatments are summarized in Figure 3.9. ANOVA revealed that the first-order rate constants for alkanes and PAHs for WSC and PBC, were not statistically different ($p = 0.399$ for alkanes and $p = 1$ for PAHs), whereas the difference in the removal rates for TEH was statistically significant ($p = 0.034$). It is interesting to note that, although the rate of degradation of total alkanes and PAHs were comparable for these two oils, at the end of the experiment, greater removal was achieved for PBC (except KMZ meso treatment where removal was similar) and more residual hydrocarbons were observed for WCS. KC samples showed no loss in alkanes, PAHs, hopane as well as TEH content (Figure 3.10).

Hopane compounds are pentacyclic triterpenes and, owing to their recalcitrant nature, are often used as conservative biomarkers. In this study, however, treatments containing the KMZ meso enrichment showed depletion in $17\alpha$(H), $21\beta$(H)-hopane concentrations. This culture metabolized almost 99% of hopane in both dilbit and PBC. Although it is a rare phenomenon, biodegradation of biomarkers has been reported previously (Frontera-Suau et al., 2002; Requejo and Halpern, 1989). Requejo and Halpern reported degradation of hopane in heavily degraded tar
sands from the Pt. Arena formation in Monterey, California. and Frontera-Suaau et al. observed that the microbial cultures enriched on the soil with a history of hydrocarbon impact were able to degrade hopane and other biomarkers at warmer temperatures. Both KMZ and AF cultures were obtained from the sediments contaminated with crude oils but only the KMZ consortium was able to metabolize at 25 °C hopane present in dilbit and PBC.

Experiments were carried out at 5 and 25°C simulating biodegradation in winter and summer conditions, respectively. The intensity of biodegradation was much lower at colder as the rate and extent of degradation were lesser when compared to 25 °C results (Figure 3.9). Similar observations have been reported previously (Atlas and Bartha, 1972; Atlas, 1975; Campo et al., 2013; Margesin and Schinner, 2001; Venosa and Holder, 2007; Zhuang et al., 2016). Extended acclimation period and slower rates of degradation at lower temperatures can be due to reasons such as decrease in solubility, crystallization of hydrocarbons, and lower metabolic rates.

Along with the temperature, microbial enrichment had a substantial influence on the degradation of both crude oils. Significant differences were noted in the nature and extent of hydrocarbon metabolism of the three cultures. All the cultures were able to degrade alkanes, while for PAH and TEH degradation, KMZ cryo culture was less effective than KMZ meso culture. The AF culture behaved differently at 5 and 25 °C. Before metabolizing the hydrocarbons, it took almost 8 days for this culture to adapt to the colder temperature. The differences in microbial activities can be explained by their composition. As discussed earlier, all three cultures were mixture of various microbial species and their composition was diverse. Diversity in the composition can be explained by difference their origin (Kalamazoo River vs Ohio River) as well as the carbon source on which they were enriched (dilbit vs ANS 521). Although studies have
reported degradation of crude oils by aforementioned dominant microbial communities (Cao et al., 2009; Jurelevicius et al., 2013; Margesin and Schinner, 2001; Rojo, 2009), AF was less competent.

This study showed that dilbit can be biodegraded, but under similar conditions, conventional crude oil was eliminated more effectively due to the higher content of lighter hydrocarbons. The potential of microbial enrichment to degrade crude oil was highly influenced by temperature as well as the composition. Well-known oil degraders metabolized both oils but their performance varied.

3.5 References


19. USEPA Dredging Begins on Kalamazoo River, Enbridge Oil Spill, Marshall, Michigan, United States Environmental Protection Agency. **2013a**.


Table 3.1: Summary of experimental layout

<table>
<thead>
<tr>
<th>Test</th>
<th>Temperature</th>
<th>Treatment</th>
<th>Sampling Events</th>
<th>Sample Replicates</th>
<th>Total Experimental unit (EU)</th>
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<td>5°C</td>
<td>WCS or PBC</td>
<td>12</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>5°C</td>
<td>Kill Control</td>
<td>1</td>
<td>3</td>
<td>3</td>
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<td></td>
<td></td>
<td></td>
<td>Subtotal EU's 39</td>
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<tr>
<td>3</td>
<td>25°C</td>
<td>WCS or PBC</td>
<td>12</td>
<td>3</td>
<td>36</td>
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<tr>
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<td><strong>Total EU's for one type of crude oil and culture 78</strong></td>
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</table>
Table 3.2: Initial microbial community structure

<table>
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<tr>
<th>KMZ -Cryo</th>
<th>KMZ-Meso</th>
<th>AF</th>
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</tr>
<tr>
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<td>p__Proteobacteria</td>
<td>p__Proteobacteria</td>
</tr>
<tr>
<td>p__Actinobacteria</td>
<td>p__Actinobacteria</td>
<td>p__Bacteroidetes</td>
</tr>
<tr>
<td>p__Bacteroidetes</td>
<td>p__Bacteroidetes</td>
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</tr>
<tr>
<td><strong>Taxon: Genus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g__Rhodococcus</td>
<td>g__Pseudomonas</td>
<td>g__Acinetobacter</td>
</tr>
<tr>
<td>g__Hydrogenphaga</td>
<td>g__Rhodococcus</td>
<td>g__Hydrogenphaga</td>
</tr>
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<td>g__Pseudomonas</td>
<td>g__Hydrogenphaga</td>
<td>g__Sphingobium</td>
</tr>
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<td>g__Xanthomonadaceae</td>
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<td>g__Parvibaculum</td>
<td>g__Pseudoxanthomonas</td>
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<td>g__Reyranella</td>
<td>g__Parvibaculum</td>
</tr>
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<td>g__Chryseobacterium</td>
<td>g__Rhodanobacter</td>
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</tr>
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<td>g__Mycobacterium</td>
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</tr>
<tr>
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<tr>
<td>g__Sphingobium</td>
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<tr>
<td>g__Janthinobacterium</td>
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Figure 3.1: Biodegradation of total alkanes for WCS (A, B) and PBC (C, D) at 5 ºC (A, C) and 25 ºC (B, D)
**Figure 3.2:** Time series concentrations of pristane and phytane (PR+PH) for WCS (A, B) and PBC (C, D) at 5 °C (A, C) and 25 °C (B, D)
Figure 3.3: Biodegradation of total PAHs for WCS (A, B) and PBC (C, D) at 5 °C (A, C) and 25 °C (B, D)
Figure 3.4: Biodegradation of naphthalene (NAP) homoluges (A,B), phenantheren (PHE) + fluorene (FLU) homoluges, dibenzothiophenes (DBT) homologues, napthbenzothiophene (NBT) homologues, and pyrene (PYR) + chrysene (CHY) homologues at 5 ºC for WCS (A, B) and PBC (C, D) using AF (A, C) and KMZ (B, D)
Figure 3.5: Biodegradation of naphthalene (NAP) homologues (A,B), phenantheren (PHE) + fluorene (FLU) homologues, dibenzothiophenes (DBT) homologues, napthbenzo thiophene (NBT) homologues, and pyrene (PYR) + chrysene (CHY) homologues at 25 °C for WCS (A, B) and PBC (C, D) using AF (A, C) and KMZ (B, D).
Figure 3.6: % removal for individual PAH compound at 5 °C (A) and 25 °C (B)
Figure 3.7: Time series concentration of TEH for WCS (A, B) and PBC (C, D) at 5 °C (A, C) and 25 °C (B, D)
Figure 3.8: Time series concentration of hopane for WCS (A, B) and PBC (C, D) at 5 °C (A, C) and 25 °C (B, D)
Figure 3.9: Summary of rate coefficients for total alkanes (A), total PAHs (B), and (C) TEH for all treatments.
Figure 3.10: Comparison between day 0 and KC concentrations of total alkanes (A), Total PAHs (B), TEH (C), and Hopane (D)
Chapter 4: Summary, Conclusion, and Future Work

Due to the increased production and the usage of diluted bitumen, its transportation and consequently the risk of accidental spill is also growing. Hence, the fate and the behavior of dilbit in the environment is a topic of interest. Dilbit exhibits quite distinct properties as compared to conventional crude oils, yet no conclusive results have been reported regarding the ease and extent of its biodegradability. Thus, the main focus of this research was to assess the biodegradation of diluted bitumen with varying temperature and microbial community, and also comparing it to the biodegradability of a conventional crude oil under the same conditions.

To achieve this goal, various bench scale experiments were run in freshwater media. Two types of dilbits, Western Canadian Select (WCS) and Cold Lake Blend (CLB), were evaluated to account for the variability in dilbit products. These dilbits were characterized before the degradation studies. Although they were produced from bitumen obtained from different deposits, no significant differences were observed in their physical and chemical properties. Both dilbits were degraded by microbial culture obtained from the dredging operations on the Kalamazoo River after the Enbridge Energy Spill. The rate as well as the degree of removal for two dilbits were comparable and hence, for further investigation only WCS was used.

As the previous experiment revealed that the diluted bitumen can be biodegraded, the next step was to compare its biodegradability with a conventional crude oil. Biodegradation experiments under various conditions showed that the rate of degradation of dilbit (WCS) and conventional crude oil (PBC) were very much alike, but PBC degraded to a greater extent and higher quantities of residual heavy hydrocarbons were noted in the case of dilbit. The present work concluded that higher concentrations of lighter hydrocarbons in conventional crude oil facilitated
the higher removal whereas for diluted bitumen, presence of heavier hydrocarbons like resins and asphaltenes hampered the biodegradation.

The aforementioned studies were carried out at 5 and 25 ºC to simulate winter and summer conditions. Temperature influenced the degradation of dilbit significantly. Rate and extent of degradation at 25 ºC were much greater as compared to 5 ºC. Thus, it can be concluded that the warmer temperatures were optimal for dilbit degradation whereas at lower temperature, dilbit metabolized, but the process was slow and extent of removal was lower.

These biodegradation studies were carried out using three microbial consortia, Kalamazoo River enrichment (KMZ) grown at 5 ºC (cryo) and at 25 ºC (meso) as well as Anderson Ferry enrichment (AF) which was enriched at 25 ºC. All three cultures showed the presence of diverse oil degrading bacteria and the differences in their compositions affected the biodegradation of dilbit. Extensive degradation of dilbit was seen in the treatments with KMZ meso culture, whereas KMZ cryo culture showed much slower metabolism. AF culture behaved distinctly at the two temperatures and their degradation at both the temperature was less effective. *Pseudomonas* and *Rhodococcus* genera were dominant in KMZ cultures, whereas *Acinetobacter* was dominant in AF consortia. From the biodegradation data, it can be seen that the two former genera were more effective than the latter. In-depth microbial community structure analysis is required to understand the differences in their activities.

Overall, this research provided basic evidences to conclude that the diluted bitumen was capable of undergoing microbial degradation, but removal of conventional crude oil was much effective under similar conditions. Degradation of dilbit was highly influenced by the temperature as well as the microbial communities present in the consortia.
While this study demonstrated biodegradation of diluted bitumen, further research is required, as biodegradability is influenced by various factors. Dilbit is richer in heavier hydrocarbons like resins and asphaltenes and most of these compounds were not quantified by the methods used in this study. Therefore, methodologies should be explored to evaluate effect of biodegradation on these compounds. It is observed that the dilbit weathers more rapidly as compared to conventional crude oil. Hence, biodegradation studies can be conducted with dilbits having different weathering levels. The present work reported biodegradation of diluted bitumen in freshwater. It will be worthwhile to study its biodegradation in marine environments. It is important to determine effective countermeasures in the case of an oil spill. Many techniques such as use of dispersants or sorbents have proven to be effective in case of conventional crude oil spills. Hence, measuring effectiveness of these products for dilbit clean-up will be a valuable contribution. Another worthwhile study would be the investigation of the biodegradation of biomarkers. As the degradation of 17α(H), 21β(H)-hopane was observed in the treatments using KMZ meso enrichment, it will be interesting to examine its effect on homologues of hopane and other biomarkers such as norhopane, steranes, and oleanane.